Reproductive Toxicity Test of Plant-Derived Insecticide in Male Rats

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ABSTRACT

This study evaluated the safety of bioinsecticide derived from *Stemona curtisii* Hook. F. and *Mammea siamensis* Kosterm. on reproductive system of male rat. The rats were orally administered with bioinsecticide at the doses of 2, 10 and 50 mg/kg body weight for 60 consecutive days in comparison with 2 mg/kg body weight of methomyl. It was found that rats treated with the bioinsecticide and methomyl showed a significant decrease in testis, seminal vesicle and prostate weights when compared to control rats (*p* < 0.05). Nevertheless, a decrease in epididymal sperm concentration and the histopathological alterations of the rat’s testes were found only in rats treated with methomyl, but not with bioinsecticide.

Keywords: bioinsecticide, reprotoxicity, male rat, *Stemona curtisii* Hook. F., *Mammea siamensis* Kosterm

1. INTRODUCTION

It is well accepted that widespread use of synthetic insecticides in agriculture has caused severe environmental pollution and health problems for both animals and humans. Toxic effects of synthetic insecticides on various organ system of mammals especially, reproductive system have been documented. Exposure to several insecticides has been reported to cause sexual dysfunction in male and female rats [1]. To reduce these problems, recent interest has been focused on bioinsecticides derived from plant materials as alternatives to control insect pests. Many plants species have been reported to have insecticidal properties such as *Acorus calamus* L., *Eupatorium odoratum* and *Azadirachta indica* A. Juss [2]. In Thailand, *Stemona curtisii* Hook. F. and *Mammea siamensis* Kosterm., are commonly used in agriculture for insect control. The researches on bioinsecticide using these two plant species as active constituents have been previously reported. Surangin B, a bioactive compound isolated from *M. siamensis* crude extract and several alkaloids found in *Stemona* species expressed very strong contact and antifeedant activities on diamondback moth larvae (*Plutella xylostella* Linn.) as well as repellent activity on *Spodoptera littoralis* larvae [3]. The safety evaluation of *S. curtisii* extract on mice
has been reported. There were no alterations of blood chemical values, hematological parameters and histology of internal organs of male and female mice treated with *S. curtisii* at the doses of 2 and 10 mg/kg body weight for 30 days [4]. Nevertheless, quite a lot of research has been reported on the toxicity of bioinsecticide derived from plant materials on male reproductive system. A significant decrease in the weights of testes, epididymis, seminal vesicle, testicular sperm count and epididymal sperm count were observed in rats treated with *A. indica* leaves extract [5]. However, the effect of *S. curtisii* and *M. siamensis* on male reproductive system has not been reported. The present study, thus, aimed to evaluate the safety of a insecticide-derived from *S. curtisii* and *M. siamensis* on reproductive system of male rat in comparison with methomyl, synthetic pesticide. The data obtained from this study could be useful as the reference for future study concerning the safety level of *S. curtisii* and *M. siamensis* in mammals.

2. MATERIALS AND METHODS

2.1 Phytochemical Analysis

Bioinsecticide obtained from Natural Product Research Unit, Department of Biology, Faculty of Science, Chiang Mai University, Thailand, was tested for the presence of bioactive compounds. Standard screening tests and conventional protocols were used for the determination of alkaloids, saponins, tannins, flavonoids, phenolics, cardiac glycosides, triterpenes, coumarins, phlobatannins, steroids, anthraquinones and reducing sugar.

2.2 Animals

Forty adult male albino rats (*Rattus norvegicus*) weighing 200-250 g were purchased from the National Laboratory Animal Center, Mahidol University, Salaya Campus, Nakhon Pathom, Thailand. Animals were acclimatized to the laboratory conditions for 1 week in the sanitary cages at environmental temperature 25 ± 2 °C under a 12-hour light/dark cycle. They were fed with standard commercial pellet chow and tap water *ad libitum*. All procedures involving the animals were conducted with strict adherence to guidelines and procedures reviewed and approved by the Institutional Animal Care and Use Committee of the Biology Department, Faculty of Sciences, Chiang Mai University (Re. 003/06).

2.3 Animal Treatment

The rats were randomly divided into five groups of eight animals each. Group 1 received 1 ml of distilled water via oral gavage once a day and serve as control. Animals from group 2, 3 and 4 were given bioinsecticide orally at the doses of 2, 10 and 50 mg/kg body weight, respectively. Group 5 received methomyl at a dose of 2 mg/kg body weight. Dosing of the bioinsecticide was done base on the doses used in previous study [4]. All animals were treated daily at 8 o’clock for 60 days. The individual animal body weight was recorded weekly throughout the experimental period.

2.4 Organ Weight Measurements

At the end of the experimental period, all rats were fasted overnight and sacrificed as per guidelines of our institutional animal ethics committee. The animals were quickly dissected. Reproductive and accessory organs (testes, seminal vesicle, prostate gland and epididymis) were immediately removed and weighed. The relative weight of reproductive organs (reproductive organs weight/100 g body weight) of each rats were calculated. Left testes were placed in Bouin’s fixative for histological analysis.

2.5 Sperm Concentration Determination

The concentrations of spermatozoa collected from the cauda epididymis were estimated. Briefly, the left cauda epididymis of each rat
was minced, carefully mixed with 10 ml of 0.9% normal saline solution and kept at 37 °C for 5 min for the dispersion of sperm into medium. Epididymal sperm suspension was pipetted very gently and a drop of sperm suspension was then transferred to a hemocytometer counting chamber. The counting chamber was observed under a light microscope at 40x magnification and the sperm counts were calculated.

### 2.6 Histopathological Examination

The fixed testes of rats were dehydrated with progressively increasing concentrations of ethanol. The tissues were passed through xylene solution to clear the ethanol and facilitate molten paraffin wax infiltration (55 °C). After that, they were embedded in a wax block. Paraffin sections of 6 μm thickness were cut with the rotary microtome and placed on cleaned glass slides. Finally, the sections were stained with hematoxylin and eosin. The stained slides were examined using a light microscope where the photomicrographs of the tissue samples were recorded. The histological evaluation was qualitatively conducted in 4 certain fields/section and 10 sections/rat were examined. The predominant degree of tissue damage was recorded as that occupying more than half of the field.

### 2.7 Statistical Analysis

For statistical analysis, data were analyzed by one-way analysis of variance (ANOVA) and the significance level was calculated using Duncan test. The statistical Package of Social Sciences (SPSS) software version 16 for Windows was employed. The results were expressed as mean ± standard error (SE). A level of P value less than 0.05 was considered to be significant.

### 3. RESULTS AND DISCUSSION

The results of our study showed a significant decrease in weights of testes and accessory sex organs of bioinsecticide and methomyl-treated rats when compared to those of controls (Table 1). Moreover, a decrease in epididymal sperm concentration of rats treated with methomyl at a dose of 2 mg/kg body weight was found (Table 2). Reduced epididymal sperm number in adult animals indicated that there has been certain interruption in the process of spermatogenesis. Many insecticides have been reported to cause gonadal dysfunction in male. A decrease in the weight of testes and sperm count was noted in propoxur and chlorpyrifos intoxicated rats [6]. Oral administration of methomyl in rats was found to decrease the fertility index, weight of testes and accessory male sexual glands, serum testosterone level and sperm motility and count [7]. Histopathological observation revealed that administration of bioinsecticide at all doses used did not cause any alteration in testicular tissue of rats (Figure 1B-D). The disorganization and destruction of spermatogenic cells as well as the increase in seminiferous tubule diameter observed in rats treated with methomyl (Table 2 and Figure 1E) are not surprising. It has been previously reported by Mahagoub and El-Medany (2001) that chronic exposure to methomyl revealed a various degree of degenerative changes in the seminiferous tubules up to total cellular destruction [1] and various mechanisms from metabolites of methomyl were suggested as the causes of cell destruction. Based on our results, although, bioinsecticide derived from *S. curtisi* and *M. siamensis* was less toxic than methomyl, they both markedly reduced reproductive organs weight of rats. It is possible that both bioinsecticide and methomyl may alter the physiological activity of rats through the similar mechanisms. Therefore, it is important to note that bioinsecticides derived from *S. curtisi* and *M. siamensis* are likely to be toxic on male reproductive system. Methomyl is a carbamate pesticide which primarily acts on central nervous system (CNS) by inhibiting acetylcholinesterase in mammals and resulting in
Table 1. Relative weight of testes and accessory reproductive organs of rat treated with bioinsecticide at the doses of 2, 10 and 50 mg/kg body weight and methomyl for 60 days compared to the control group.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Relative organ weights (g/100g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Testes</td>
</tr>
<tr>
<td>Control</td>
<td>1.05 ± 0.000a</td>
</tr>
<tr>
<td>Bioinsecticide 2 mg/kg body weight</td>
<td>1.05 ± 0.001b</td>
</tr>
<tr>
<td>Bioinsecticide 10 mg/kg body weight</td>
<td>1.02 ± 0.001b</td>
</tr>
<tr>
<td>Bioinsecticide 50 mg/kg body weight</td>
<td>1.05 ± 0.001b</td>
</tr>
<tr>
<td>Methomyl 2 mg/kg body weight</td>
<td>0.96 ± 0.000c</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE of 8 replicates. A different superscript letters within each column are significantly different (p < 0.05).

Table 2. Sperm concentration and seminiferous tubule diameter of rat treated with bioinsecticide at the doses of 2, 10 and 50 mg/kg body weight and methomyl for 60 days compared to the control group.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Sperm concentration (x 10^6/ml)</th>
<th>Seminiferous tubules diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>79.948 ± 8.670a</td>
<td>208.3 ± 10.850a</td>
</tr>
<tr>
<td>Bioinsecticide 2 mg/kg body weight</td>
<td>68.433 ± 14.042ab</td>
<td>215.1 ± 9.580c</td>
</tr>
<tr>
<td>Bioinsecticide 10 mg/kg body weight</td>
<td>68.247 ± 8.354bc</td>
<td>215.7 ± 22.390ab</td>
</tr>
<tr>
<td>Bioinsecticide 50 mg/kg body weight</td>
<td>74.980 ± 9.560cd</td>
<td>220.5 ± 11.020cd</td>
</tr>
<tr>
<td>Methomyl 2 mg/kg body weight</td>
<td>59.236 ± 10.001bd</td>
<td>245.95 ± 16.660bd</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± SE of 8 replicates. A different superscript letters within column are significantly different (p < 0.05).

the alteration of cholinergic neurotransmission. Since hypothalamus is the important part of hypothalamic-pituitary-gonadal axis, its impairment caused by methomyl may lead to sex hormone imbalance, especially androgen deficiency [8] and consequently end up with reproductive failure. Earlier studies reported that disulfiran and dithiocarbamate, a carbamate metabolite can interfere with catecholamine neurotransmitter metabolism by inhibiting the dopamine beta-hydroxylase activity, the enzyme that converts dopamine to norepinephrine and norepinephrine then stimulates the release of gonadotropin releasing hormone [9]. This mechanism plays an important regulatory role in brain hypothalmo-pituitary luteinizing hormone release. Thus, a decrease in testis and accessory organ weights and sperm concentration found in this study may be due to androgen deficiency following anti-androgenic property of investigated insecticides. Testis, a complex organ containing several cell types such as germ cells, Sertoli cells and Leydig, functions mainly in spermatogenesis and steroidogenesis. Testicular damage induced by any factors, therefore, could certainly decrease its function. Mansour et al. (2009) reported that methomyl has the capability to induce oxidative damages resulting in tissue degeneration by increasing lipid peroxidation and perturbations in antioxidant
enzymes such as glutathione-S-transferase and superoxide dismutase [10]. Interestingly, bioinsecticide used in this study did not cause any alteration in testicular tissue of rats. It is possible that phytochemical contents presence in the bioinsecticide such as alkaloids, saponins, flavonoids, cardiac glycosides, terpenoids, coumarins and phytobatannins (data not shown) may act as antioxidants. Thus, these bioactive compounds could play a role in inhibiting lipid peroxidation by maintaining the balance between free radicals and antioxidant status in rats and consequently neutralized the toxicity of reproductive organs.

4. CONCLUSION

It was concluded that a significant alterations in testes, seminal vesicle and prostate weight of rats treated with bioinsecticide derived from crude extracts of \textit{S. curtisii} and \textit{M. siamensis} found in this study is a sign of toxic effects of bioinsecticide on reproductive system of mammalian species. Based on our results, we do not recommended the long-term use of \textit{S. curtisii} and \textit{M. siamensis} in agriculture.

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REFERENCES


